"A Study on Plant Extracts of *Zanthoxylum armatum* (DC) as Natural Food Preservatives"

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A SYNOPSIS

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IIMT UNIVERSITY MEERUT

Supervised by:

Dr. Garima Bartariya

(Associate Professor)

Department of Botany

School of Life Sciences and Technology

IIMT University Meerut. (U.P)

Submitted by:

Naresh Singh Chauhan

Research Scholar

Department of Botany

School of Life Sciences and Technology

IIMT University Meerut. (U.P)

1. INTRODUCTION:

Zanthoxylum is a genus of aromatic dioecious plants commonly known as Sichuan pepper or prickly ash, belonging to the Rutaceae family with over 200 species, it is believed as the native to various regions such as the America, India, China, Japan and Africa,known for its medicinal and culinary uses. In India and Nepal*Z. armatum*(DC) are commonlyknown as Timru, Timbru tree or Toothache plant and prickly ash. The genus isindigenous to regions of India such as Meghalaya, Mizoramand Manipur(Gupta, et al.2011).*Z. armatum* (DC) comprises a group of 11 species within its genus, notably known for their medicinal properties, namely *Z. limonella, Z. avicennae, Z. simulans, Z. rhesta, Z. nitidium, Z. armatum, Z. planispinum, Z. aconthopdium, Z. ovalifolium, Z. oxyphyllum,* as well as *Z. budrunga*, 4 species among them are indigenous to India specifically, *Z. acanthopodium, Z. armatum*(DC),*Z. Budrungaare* and *Z. oxyphyllum edgew*. These particular members, recognized for their medicinal attributes, have shown higher efficacy in treating various ailments with minimal or no adverse effects(Samant et al. 1998). *Zanthoxylum spps.* are commonly shrubs, trees, or occasionally herbs exhibit a range of growth habits, including scrambling or scandent forms, characterized by the presence of aromatic volatile oils stored in glands that are apparent on the surface of leaves, young branches, inflorescences, various parts of flowers, fruits and seed cotyledons. *Zanthoxylum alatum,* identified as *Z. armatum*(DC) categorized as a shrub or petite arboreal entity existing in the subtropical zones of the Himalayan Mountain range and various other geographical areas characterized by elevations spanning from 3500 to 7000 feet.

1.1. Taxonomic features of *Zanthoxylum*

1.1.1. Classification:

Kingdom: Plantae:

Clade: Angiosperm/ eudicots:

The botanical category Angiosperms is a clade in the Plantae kingdom. A taxonomic group known as Eudicots falls within the clade of Angiosperms. Eudicots also referred to as "true dicots" belong to a clade of angiosperms distinguished by the presence of two seed leaves or cotyledons.

Order: Sapindales:

Sapindales an order of angiosperms, encompasses economically significant families like Rutaceae, Sapindaceae and Anacardiaceae.

Family: Rutaceae

The Angiospermic family Rutaceae is widely known as the citrus or rue family comprises a diverse array of fragrant plants, many of which yield essential oils. The Rutaceae Juss. is classified within the order Sapindales primarily due to the presence of specific charectristics such as leaves they

are pinnetely compounds, in the bark and leaf vains as well as in wood rays resin ducts are absent, the presence of triterpenoid compounds and flowers with a noticeable nectar disk(Judd et al., 2009). The family comprises species that hold considerable economic values, utilized as decorative florafor medicinal or culinary applications and within the fragrance sector, encompassing varieties like lemon, orange, kumquat, mandarin lime as well as grapefruit.

Genus: *Zanthoxylum*:

Prominent species under the genus *Zanthoxylum*: *Zanthoxylum piperitum* identified as Japanese prickly ash or Sichuan pepper, *Zanthoxylum bungeanum* is another species often known as sichuan pepper. *Zanthoxylum armatum*(DC) also referred to as Indian prickly ash. *Zanthoxylum americanum* recognized as the American prickly ash, also indigenous to North America. *Zanthoxylum ailanthoides* also recognised as winged prickly ash or Japanese prickly ash, originating from East Asia.

1.2.1. Identifying characteristics of Zanthoxylum:

Habit:*Zanthoxylum* species are generally shrubs or small to medium-sized trees. Zanthoxylum *coco* isan example of tree.

Habitat:*Zanthoxylum* species can often be found on hillsides and mountain slopes, tropical and subtropical regions. Some species are adapted to dry, rocky soils and can tolerate less fertile conditions.

Root:*Zanthoxylum* is characterized by the presence of a taproot system, typically woody in nature and sometimes emitting aromatic compounds, indicating the existence of a central primary root that extends deeply into the soil. The taproot displays considerable thickness and strength, playing a crucial role in anchoring the plant firmly in place.

Stem: The stems of *Zanthoxylum* typically exhibit a woody nature. They are characterized by their hard and long-lasting wood, particularly evident in mature specimens. An identifying features of *Zanthoxylum* stems is the existence of pointed prickles or thorns, which may differ in dimensions and concentration based on the particular species.

Leaves: The leaves*Zanthoxylum* species are positioned alternately, with leaflets that are odd-pinnately segmented into 3 or more or occasionally displayed in a digitate fashion with 3 leaflets.

Thorns: Prescence of thorn in *Zanthoxylum* species are one of the most distinguishing features, many species exhibit thorns or spines on stems, branches, and leaf petioles. Plants bearing spines, thorns, or prickles have undergone evolutionary developments as a means of protecting themselves against herbivorous animals(Airoldiet al.,2020).Spines represent adaptations of leaves, thorns are alterations of stems, and prickles are modifications of epidermal cells (Huchelmann et al., 2017).

Flowers: Generally small, usually unisexual, small, and occur in inflorescences. They have five petals and sepals.

Fruit:*Zanthoxylum* species has fruits is type of capsules or drupes, displaying vibrant hues and enclosing shiny black seeds. The infructescence of *Zanthoxylum* is commonly interpreted as a cluster

of individual fruits, highlighting the critical need for accurate identification methods (Dan et al., 2024).

1.2.2. Anatomical Description

Study of internal structure of plants is known as anatomy. Anatomy is one of the major sources or keys of identification of taxa. In *Zanthoxylum* species, the leaflets have a dorsiventral structure. When you look at a cross-section, the upper surface (adaxial) has larger, more uniformly shaped cells compared to the lower surface (abaxial). The stomata, which are openings for gas exchange, are found on the lower surface and are level with the surrounding cells. The leaflets have secretory cavities only along the edges. The vascular bundles, which transport water and nutrients, are arranged in a collateral manner, small and close together and are surrounded by a protective bundle sheath. Both the upper and lower surfaces of the leaves have a single layer of epidermal cells with a cuticle layer. The mesophyll cells also contain collateral vascular bundles, surrounded by a bundle sheath. Secretory cavities are found throughout the leaflets, including at the edges (Antonia J. Oggero et al., 2016).

In *Zanthoxylum* species, the leaflets have a dorsiventral structure. When you look at a cross-section, the upper surface (adaxial) has larger, more uniformly shaped cells compared to the lower.

Anatomical investigations conducted on different *Zanthoxylum* species elucidate intriguing characteristics. The leaf structure of *Zanthoxylum caribaeum* showcases an unstratified epidermis, glandular trichomes a hypostomatic leaf with anomocytic stomata and a dorsiventral mesophyll containing secretory cavities that produce lipidic and mixed secretions. Similar features are observed in *Zanthoxylum macrophylla* across leaf, stem, petiole and root transverse sections with paracytic stomata exclusively found on the abaxial leaf surface. *Zanthoxylum armatum* (DC) displays distinctive anatomical traits in its leaves, stem bark and fruit. The compound leaves are bifacial and punctate, featuring a single-layer epidermis, devoid of trichomes and housing oil cavities. Stomata exhibit a brachparatetracytic, actinostephanocytic and straucytic nature with stomatal clusters located on the lower epidermis. The stem bark and fruit demonstrate varying tissue organizations with the seed lacking endosperm and containing an elongated embryo.

Root anatomy encompasses a variety of structural elements, comprising the epidermis, exodermis, fibrous layer, cortical parenchyma, endodermis, pericycle and pith, characterized by unique attributes like suberin lamellae, lignified cells, Casparian strips and sclerified tissues. Alkaloids and lignans are present in the roots of *Zanthoxylum* species.

The stem anatomy of *Zanthoxylum* species unveils significant features. Species such as *Z. macrophylla* and *Z. armatum*(DC) demonstrate resemblances in stem anatomy, particularly in the presence of rays in the secondary stem and root. *Z. armatum*(DC) stems possess armed structures and secretory cavities, contributing to their distinct characteristics. Meanwhile, the stem anatomy of *Z. caribaeum* is defined by an unstratified epidermis, glandular trichomes and secretory cavities in the mesophyll that produce lipidic and mixed secretions.

1.3. Phytoconstituents of *zanthoxylum*:

Zanthoxylum species harbor a multitude of chemical constituents exhibiting a wide range of properties and functionalities. Phytochemical components of *Zanthoxylum armatum*(DC) fruit is abundant in bioactive secondary metabolites. The presence of these different types of metabolites in the *Zanthoxylum* helps give it antimicrobial properties through different mechanisms (Sharma et al., 2015). The phytochemical screening of *Zanthoxylum armatum*(DC) fruit extract revealed the presence of several compounds, including flavonoids, alkaloids, volatile oils, tannins, polyphenols, terpenoids, quinones and saponins, these phytochemicals demonstrate a diverse array of functions such as antitumor, antimalarial, antioxidant, antifungal, antibacterial, antiviral, antiinflammatory, larvicidal and antisickling properties (Ishwor et al., 2021), (Okagu2021), (Umesh et al., 2015). Essential oils, produced by aromatic plants for defensive and reproductive purposes, consist of intricate combinations of compounds with diverse levels of concentration, primarily comprising monoterpenes, sesquiterpenes and their related derivatives, alongside certain minor components that hold notable functions within specific essential oils(Zribiet al., 2019).

1.4.Utilization significance of *Zanthoxylum*:

The *Zanthoxylum* fruit are frequently utilized in various medicinal practices used in traditionally and in a variety of other contexts such as chemo preventive agents, dental hygiene and fragrances (Nooreen et al., 2019).In the realm of traditional medicine, numerous species of plants are employed to treatment of conditions such as malaria, trypanosomiasis, sickle cell anemia as well as microbial infections, encompassing enteritis and tuberculosis (Erichsen-Brown C. 1979).The stem barkand roots are used to relieve pain and treat conditions like arthritis, leprosy, stomachachesand sexually transmitted diseases. In Cameroon as documented by (Burkill 2000 and Ngoumfo et al., 2010). Many components of *Zanthoxylum* species have been traditionally employed in medicinal practices to alleviate tooth pain, respiratory infections, gastrointestinal discomfort and also to counteract venomous snake envenomation(LOU J. R., 2018).Moreover, there has been scientific investigation into the potential of *Zanthoxylum* for the discovery and formulation of novel agents targeting tumor growth (Yuan H. M. et al.,2021).The distinctive fragrance and taste of *Zanthoxylum* pericarps have garnered popularity as culinary spices, primarily attributed to the tingling effect they induce in the oral cavity (Pan et al., 2017).

The genus *Zanthoxylum* is of considerable economic importance due to its diverse applications in the culinary, medicinal and agricultural domains. *Zanthoxylum* species are under scrutiny for their pharmacological potential, exemplified by the ACE inhibitory peptides isolated from *Zanthoxylum bungeanum* seed protein hydrolysates, which could offer advantages in addressing hypertension (Wu et al., 2016).

1.4.1 Culinary Applications

In China and various regions of Eastern Asia, *Zanthoxylum bungeanum* (also known as *Zanthoxylum piperitum* Benn.) is extensively employed as a culinary seasoning due to its purported health advantages (Hwang et al., 2018) and also utilized in skincare products to uphold skin integrity (Hwang et al., 2020).

In Japan, the leaves, fruits and bark are used to treat bacterial and fungal infections. They are also used in cooking and for preserving food (Hatano et al., 2004). Likewise, different parts of *Z. schinifolium* are used as spices in cooking and for treating discomfort of stomach, jaundice, diarrhea and the common cold seen in Eastern Asia (Cui et al., 2009).

Zanthoxylum species such as *Zanthoxylum piperitum* and *Zanthoxylum bungeanum*hold significant value within various culinary traditions, particularly in the realm of Asian gastronomy,referred to commonly as Sichuan pepper, these particular species play a crucial role as key components in numerous traditional dishes across different regions. Through the utilization of the dried fruit husks, a distinct flavor profile is introduced, characterized by a unique sensation of tingling or numbing, thereby elevating the overall taste and sensory perception of the cuisine. The utilization of these species in culinary practices carries substantial economic consequences, influencing both local and global spice markets.

1.4.2 Medicinal Uses:

The genus *Zanthoxylum* occupies a prominent position in traditional medicinal practices observed in diverse cultural settings. Numerous species are employed for their therapeutic attributes, encompassing analgesic effects for pain relief, anti-inflammatory actions to mitigate inflammation, antimicrobial characteristics demonstrating antibacterial and antifungal properties and digestive aid properties that enhance digestion and manage gastrointestinal ailments. These therapeutic applications form a significant foundation of the traditional and herbal medicine sectors, supporting the livelihoods of herbal remedy practitioners and suppliers.

Plants have been recognized as valuable reservoirs of nutrients and medicinal compounds utilized for the treatment of various health ailments across diverse regions globally. The stem bark is traditionally used for its antiinflammatory, digestive, stomachsoothingand antiparasitic properties. It has also shown to inhibit the growth and replication of human keratinocytes (Verma et al., 2021)and hepatoprotective properties (Sabir et al., 2017), (Kalyankumarrajuet al., 2021).

1.4.3. Agricultural and Ecological Value in rural communities:

Zanthoxylum species play a crucial role in sustainable agricultural practices and ecological equilibrium. Their utilization in agroforestry systems aims to enhance soil quality, prevent erosion and create habitats for beneficial insects and birds. The adaptability of these species to diverse environmental conditions underscores their significance in reforestation and land reclamation initiatives, ultimately fostering biodiversity and bolstering ecosystem resilience. The cultivation and harvesting of *Zanthoxylum* species significantly contribute to the economic well-being of rural communities in many regions. The commercialization of products such as Sichuan pepper derived from these species plays a vital role in supporting local economies, facilitating community growth and improving the livelihoods of small-scale farmers and traders.

1.4.4. Industrial Applications:

The industrial sector benefits from the essential oils obtained from *Zanthoxylum* species particularly in sectors such as cosmetics and perfumery. These oils, prized for their aromatic qualities, serve as key components in perfumes, soaps, and skincare products. Moreover, they find application for natural flavoring agents.

1.4.5. Cultural Significance:

Beyond their economic importance, *Zanthoxylum* species hold cultural and ceremonial significance in various societies. Frequently featured in traditional rituals, these species symbolize concepts of protection and purification, solidifying their integral role in the cultural and economic tapestry of these communities. Nevertheless, the economic significance of *Zanthoxylum* faces challenges from diseases that impact both yield and quality. Specifically, the emergence of yellow-flower disease in *Zanthoxylum schinifolium* has resulted in substantial economic setbacks for cultivators due to diminished harvests (Xu et al., 2021). Correspondingly, bacterial fruit black rot disease affecting *Zanthoxylum armatum*(DC) has led to decreased yields and compromised quality (Liu et al., 2021).

2. Literature Review:

The medicinal attributes of plants have been acknowledged since ancient eras, as individuals have depended on the natural world for the management of ailments. The practice of utilizing medicinal plants for healing dates back to the origins of humanity. The extraction of medicine from plants can be derived from a variety of components of plants, such as leaves, roots, flowers, root, stem etc. These plant parts harbors different bioactive compounds. In India and other developing countries, people use medicinal plants for their healing properties to treat different illnesses (Verma et al., 2021).

A majority of the community associates spiritual importance and mystical attributes with the tree.The Bhotiya community in the Uttarakhand region has established a system for sharing and distributing Timur to villages and people in areas where it doesn't naturally grow,in return, they receive millets, cereals, pulsesand fruits that are not grown in Bhotiya villages along with other items like woolen clothing. This mutual exchange is maintained even among individuals who have relocated from the mountains and now reside in distant urban centers and towns.

Wen J.et al., (2024),worked on "Pharmacological activities of *Zanthoxylum L.* plants and its exploitation and utilization". Extracts and compounds from *Zanthoxylum L.* such as essential oils, alkaloids, amides, flavonoids and phenylpropanoids, have antiinflammatory effects.Amide compounds from *Zanthoxylum L.* are the key ingredients that help in treating with inflammatory pain.They also suggestednumerous dangerous microbes and parasitesincluding bacteria, fungus, viruses and parasites are the cause of infectious diseases. *Zanthoxylum L.* is made up of a variety of pharmacologically active substances, some of which or their monomer components have insecticidal, antiviral, and antibacterial qualities that help them fight infections.

Wang Z.J. et al., (2024),worked on "Antimicrobial ingredients of *Zanthoxylum motuoense* and potential in fresh pork meat preservation" and reported total of 21 phytochemicals as the major bioactive compounds isolated from *Zanthoxylum moutons* extracts. They were crucial in inhibiting microbial biofilm formation and demonstrating strong antimicrobial activity against foodborne pathogens like *C. albicans* and *Methicillin resistant Staphylococcus aureus*. They also found bioactive compounds isolated from *Zanthoxylum motuoense* extracts were found to be effective in preserving fresh pork meat by inhibiting microbial growth and preventing spoilage. These compounds offer a natural alternative to synthetic additives with potential health risks.

Kaigongi M. M et al., (2023),studied on "A versatile untargeted metabolomics-driven technology for rapid phytochemical profiling of stem barks of *Zanthoxylum* species with antioxidant and antimicrobial activities" And They found that the presence of saponins, alkaloids,flavoextrnoids, tannins, anthraquinones and terpenoids, in the plant extracts contributed to their effectiveness as potent antimicrobial agents. These phytochemical compounds contribute to the antimicrobial activities of the extracts, enhancing their efficacy in inhibiting microbial growth. The plant samples tested in that study exhibited potent antimicrobial activities that were significantly comparable to commercial antibiotics

at the tested concentration levels. Particularly, the plant acts showed strong potency against *E. coli*, a key indicator of water pollution.

Ramet al., (2023),worked on "Analysis of antibacterial and antioxidant activities of leaf and bark extracts of *zanthoxylum armatum*(DC)". In their investigation, they tested the antibacterial activity against four human pathogenic microorganisms includes *Shigella dysenteriae*, *Salmonella typhi*, *Listeria monocytogenes* and *E- coli*, using the Agar-well diffusion method. The results indicated that methanol extracts had larger zones of inhibition against *E- coli*, while acetone extracts showed stronger inhibition zones against *Shigella dysenteriae* (16.67 mm) and *Salmonella typhi* (16.67 mm) for the bark and leaf extracts, respectively. The bark extracts showed greater antioxidant capacity across the leaf and bark extracts.

Yang J. et al., (2022),studied on "Chemical Composition and Antifungal Activity of *Zanthoxylum armatum*(DC) Fruit Essential Oil against *Phytophthora capsica*" Oomycetes are pathogenic fungus that severely harm fruits and vegetables all around the world. Potential prospects for the development of fungicides are plant essential oils. Their findings showed that the mycelial proliferation of Phytophthora capsici was greatly suppressed by *Z. armatum*(DC) fruit essential oil. Furthermore, *Z. armatum*(DC) fruit essential dramatically decreased *P. capsici*-caused illness symptoms in pepper and strongly inhibited the spore generation and germination of *P. capsici.* According to their microscopic and biochemical investigations, *Z. armatum*(DC) fruit essential caused *P. capsici's* ultrastructure and membrane integrity to be compromised, which resulted in cell death and leakage of cellular contents.

Yang J.et al., (2022),worked on "Chemical Composition and Antifungal, Anti-Inflammatory, Antiviral and Larvicidal Activities of the EssentialOils of *Zanthoxylum acanthopodium*. from China and Myanmar" Their finding suggested that the volatile compounds present in the *Zanthoxylum acanthopodium* fruits collected from Myanmar and China were identified to be abundant in terpinen-4-ol and limonene as well as β-myrcene. These compounds effectively inhibited four types of human skin fungi and showed significant anti-inflammatory effects by reducing the level of NO in RAW264.7 cells,and they also find the fungicidal efficacy of the volatile compounds.

Wu et al., (2021),studied on "Network pharmacology□based analysis of the underlying mechanism of Huajiao for pain relief" looked into and clarified the mechanism behind *Z. bun*geanum's analgesic action using network pharmacology. Their findings suggest that the pain-relieving effect is regulated by three main signaling pathways, work together to produce its analgesic effects.

MaY. et al., (2021), worked on "Volatile oil profile of prickly ash (*Zanthoxylum*) pericarps from different locations in China". In this study, they identified 47 compounds in the volatile oils extracted from pericarps of prickly ash.The most common compounds found were D-limonene, and linalool as well as alpha-myrcene. Volatile oil's property in pericarps of prickly ash changed depending on the plant species and environmental conditions. These environmental factors influencing the variations in volatile profiles among different *Zanthoxylum* species were diverse.

Sandulachi et al., (2021),worked on "Controlling the risk of *Bacillus* in food using berries". Research shows that adding berries to food products can lower the pH level and water activity,

creating conditions that make it harder for *Bacillus* bacteria to grow. This effect is due to the natural compounds in berries, like antioxidants and organic acids, which help preserve food. The study also looked at how adding berries affects the development of ropiness disease in wheat bread during storage. It found that berries can reduce this spoilage, showing their role as natural preservatives in baked goods.

de Andrade et al., (2020),studied on "Leaf anatomy and histochemistry of secretory structures of *Zanthoxylum caribaeum* Lam. (Rutaceae)"Acoording to their study, analysis of the leaf structure and the recognition of excretory features within the leaves of *Zanthoxylumcaribaeum*. Glandular trichomes, extrafloral nectaries and secretory cavities were observed in the leaves. The substances discharged from these cavities were ascertained to exhibit a lipidic quality comprising neutral lipids and aromatic compounds these discoveries furnish valuable insights for forthcoming taxonomical examinations of the genus.

Singh A. et al., (2020), studied on "Biological activities and Phytochemical analysis of *Zanthoxylum armatum*(DC)". and "leaves and bark extracts collected from Kumaun region, Uttarakhand, India" Their findings suggest that *Zanthoxylum armatum*(DC) extracts can be used for food preservation, extending the shelf life of food materials. This could be particularly beneficial in the food industry to maintain the quality and freshness of products.

Pang W. et al., (2019),worked on "Anticancer activities of *Zanthoxylum bungeanum* seed oil on malignant melanoma". They reported that recent research has extensively studied the roots, stems, leaves, and fruits of the natural *Zanthoxylum* plant for their anti-tumor properties.The results show that extracts from the Zanthoxylum plant and its components have strong anti-tumor effects and can effectively stop the growth and spread of various tumor cells.

Santhanam R. K.et al., (2018),studied on "Inhibition of UVB□induced pro□inflammatory cytokines and MMP expression by *Zanthoxylum rhetsa* bark extract and its active constituent hesperidin" And they sggestedHesperidin, a flavonoid extracted from *Z. rhetsa*, has been found to possess anti-inflammatory properties when tested on HDF cells exposed to UVB radiation ranging from 290–320 nm.

Zhao et al., (2017),studiedon "Antioxidants and α-glucosidase inhibitors found in "Liucha" (*Sibiraea laevigata's* young leaves and shoots of Sibiraea laevigata).and **Meng et al., (2020).**Study on "Phenolic chemical constituents of *Zanthoxylum schinifolium* pericarps and their anti-oxidative effects" They discovered 12 different polyphenol glycoside compounds from *Zanthoxylum bungeanum*.Using the ABTS+ free radical scavenging test, they evaluated the antioxidant abilities of these compounds and found that four of them from *Z. bungeanum* had strong antioxidant properties.

Li P.et al., (2015),worked on "Optimizing the extraction of polysaccharides from *Zanthoxylum bungeanum* using RSM and studying its antioxidant activity", performed an extensive evaluation of the antioxidative capacity of *Z. bungeanum* extract using a variety of assays, the results showed that the polysaccharides in the *Z. bungeanum* extract have strong antioxidant properties.

KalaC. P. et al., (2005),worked on "Traditional uses and conservation of timur (*Zanthoxylumarmatum* DC.)" through social institutions in Uttaranchal Himalaya, India. Andthey report*Z.armatum*(DC), being exhibits a diverse array of conventional applications. In Nepal, Z. *armatum*(DC) is commonly utilized in the preparation of soups and sauces. Timur is used to treat common conditions like toothaches, colds, coughs, and fevers because it is believed to produce warmth in the body.For a toothache, a fresh or dried fruit is placed on the painful tooth and kept there until its strong flavor fades. The young shoots of timur function as tools for oral hygiene. More recently, there has been a growing trend in using a powder derived from the dried fruit for dental care. Digestive issues are commonly addressed with the consumption of timur-infused soup.The Bhotiya community engages in the production of alcoholic beverages using timur, albeit the final product is only enjoyable for individuals with a strong dependency.

India possesses approximately 4.5 million plant species, with thousands of them being purported to exhibit medicinal properties for combating human diseases. The utilization of medicinal plants by traditional healers for treating various ailments spans centuries; nevertheless, there has persistently existed a query within scientific communities regarding their therapeutic efficacy. Consequently, so many studies have been done to investigate pharmacological activity of numerous medicinal plants, the majority of these plants have yet to be thoroughly examined for their phytochemical composition and pharmacological effects.

3.Research gap:

According to available literature, current investigation establishes that the complete *Zanthoxylum armatum* plant could potentially serve as a valuable resource for herbal antioxidants, natural food preservatives, anti-inflammatory agents, pharmaceuticals, and natural antibacterial compounds (against *E. coli S. aurieus*) after following thorough clinical evaluation.

As on available literature and their suggestions*Zanthoxylum armatum*(DC) extracts can be used for food preservation, and extending the shelf life of food materials. This could be particularly beneficial in the food industry to maintain the quality and freshness of products.

As per gathered literature the selected plant has such bioactive compounds which can be used to enhance the antimicrobial and antibacterial properties of those microorganisms that are responsible for meat spoilage. The current research objective is set to screen such phytoconstituents that can be used as food preservatives in vegetarian as well as non-vegetarian food products.

As with other objective-based studies, once the preservative efficiency of the bioactive components is identified, it will be essential to evaluate their antimicrobial activity.

Study the properties of anti-inflammatory and antioxidant activity of such compounds that can be used as preservatives, becomes essential to check more potential of identified bioactive compounds.

4. Objectives:

1. Preparation of aqueous and ethanolic extract from leaves and fruits of *Zanthoxylum armatum* (DC).

2. Isolation of food spoilage microbes in different categories of foods i.e. dairy andmeat.

3. Estimation of anti-microbial properties of the extracts against isolated food spoilage bacteria and fungi to check its potential as natural preservatives.

4. Assess the efficacy of plant extracts as food preservatives for dairy, vegetables, fruits and meat.

5. Screening of phytochemical properties present in foliar extract of *Zanthoxylum armatum* (DC).

5. Methodology:

5.1. Material Collection:

Material from the plants will collect from different parts (leaves, stem, bark, seeds, roots, thorn etc.) of *Zanthoxylumarmatum* (DC) from selected locations with different elevations (Dehradun and Uttarkashi district of Uttarakhand). locations of collection site and environmental conditions with season will be documented.After that plant samples were authenticate by Taxonomists, deposit voucher specimens in a recognized herbarium for future reference.

5.1.1 Estimation of anti-microbial properties against food spoilage bacteria and fungi:

(a.) Preparation of material:

AfterClean-up process (to remove any foreign matter), samples are air dry at room temperature without sunlight, and after that grind the material to fine powder with the using of mechanical grinder then store the powdered samples in air tight containers and protect from light and moisture. The material can be extracted using the cold percolation method with different organic solvents of varying polarity.

(b.) Extraction: Soxhlet Extraction:

Soxhlet extraction is one of the methods, used for the isolation of phytoconstituents. This method involved preparing of crude plant extracts with using different types of solvents like petroleum ether, acetone, chloroform as well as aqua solvent using for approximately 20 hours. Approximately 10-50 grams of desiccated and pulverized plant material take and subsequently, introduce the material into a thimble, ensuring the top is securely sealed with glass wool or cotton to prevent any material from escaping. And then Construct the Soxhlet Apparatus, begin by inserting the thimble, containing the plant material, into the primary chamber. Proceed to affix the Soxhlet extractor to a round-bottom flask filled with 150-300 ml of the designated solvent,and after it attach a condenser to the apex of the Soxhlet extractor, ensuring all connections are airtight and devoid of any leakage.

Process: Position the round-bottom flask on a heating mantle or within a water bath. Gradually apply heat to gently warm the solvent, leading to its evaporation, condensation within the condenser and subsequent return into the Soxhlet extractor. The solvent will perpetually circulate through the plant material, facilitating the extraction of pertinent compounds. Allow the extraction to continue for a duration of 6-24 hours, depending on plant material's nature, the desired compounds ensuring the solvent cycles through 5-10 times within this timeframe. After the completion of the extraction process,allow the

apparatus to cool down. Transfer the liquid extract from the round-bottom flask into a sanitary receptacle, and then into the evaporator flask. Employ the rotary evaporator to eliminate the solvent under reduced pressure and at a low temperature, resulting in a concentrated extract. Subsequent to solvent removal, deposit the concentrated extract into a pre-weighed vessel. Subject the extract to desiccation within a desiccator to eliminate any residual moisture. Finally, quantify the desiccated extract and place it in a pristine container.

(c.). Fungi:

Sample Collection: Processed food samples collect specifically, which were suspected to have fungal contamination. This initial step ensured that the samples were relevant for the study of spoilage fungi.

Isolation of Spoilage Fungi: The spoiled portions of the collected food samples were transferred using sterile forceps into Petri plates containing Sabouraud Dextrose Agar (SDA), which serves as the growth medium for fungi. This step was crucial for providing an environment conducive to fungal growth.

Incubation: The Petri dishes were incubated at a controlled temperature of approximately 25-27 °C for 5 days. This incubation period allowed the fungi to grow and form visible colonies, which could then be identified and analyzed.

Pure Culture Maintenance: After the initial growth, a pure culture of each type of colony was obtained by transferring the colonies to new SDA plates. This process helps isolate specific fungal strains for further study and ensures the cultures are free from contamination.

Identification of Fungi: The isolated cultures are identified on the basis of their morphology, as well as the coloration of the colonies. Techniques such as slide culture were employed for accurate identification, following established methods in mycology.

(d.) Antifungal Screening:

To test the antifungal activity of the plants and their parts, we can used Poisoned Food Technique (Sinha et al., 1993). In this process plant extracts to an SDA medium, then poured 20 ml of this mixture into each Petri dish and let it solidify.Then, place the selected fungal samples in the center of each dish and incubate them at 25 ± 2°C for ten days. This experiment will repeat three times. dish without plant extracts show a negative control and with sodium benzoate show as a positive control. Measurement of the growth of fungi by taking two diameter measurements at right angles every 24 hours, until the control plate was completely covered, this method used by (Mohanka R.et al., 2014).

(e.) Bacteria:

Thebacterial samples will be collected from spoilage food material. The collected samples will be cultured on Nutrient Agar media (after preparation) and it will be incubated at 37□ for 24 hours by dilution streaking into 4-quadrants. and after that identification process will be done on the basis of their morphological (shape, size, color), and food spoilage activity.

(f.). Antibacterial screening:

Agar well diffusion method can be used for antibacterial activity of the extracts. (Balouiri M.et al., 2016), (Javedet al., 2016) first prepared a nutrient agar plate,then spread different bacterial strains with the help of sterile spreader. and then we will use a borer with 8mm diameter, cut wells into the agar. After this we will pour extracts of different concentrations into each well. Then Incubated the plates at 37°C for 24 hours. After incubation, we measured the zone of inhibition (ZOI) around each well and recorded the average. We should perform this experiment three times.

5.1.2. Method employing for biochemical screening:

(a.) Qualitative method

5.1.2.1. Alkaloid testing procedures:

i)Mayer's Test: Mix 1 ml of the test solution with a few drops of Mayer's reagent (a potassium mercuric iodide solution). If a color change or precipitate appears, it indicates the presence of alkaloids.

ii) Wagner's test- When we mix 1 mL of the extract with 1 mL of potassium iodide (Wagner's reagent) and shake it, a reddish-brown precipitate means alkaloids are present.

iii) Tannic acid Test-Mix 1 ml of the test solution with a few drops of 10% tannic acid solution. A color change indicates the presence of alkaloids.

5.1.2.2. Tannin detection methods:

i) Ferric chloride (FeCl3) Test: Mix about 0.5 mg of dried plant extract powder with 20 ml of water in a test tube. Boil the mixture and then filter it. Adding a few drops of 0.1% ferric chloride solution will produce a brownish-green or blue-black color, showing the presence of tannins.

ii) Gelatin Test: Mix about 1 ml of the test solution with 1% gelatin and 10% sodium chloride solution. If a white precipitate forms, it indicates the presence of tannins.

5.1.2.3. Steroid testing:

Liebermann's Buchard test: Mix 1 ml of the test solution with acetic anhydride, boil it, and let it cool. Then add concentrated sulfuric acid. If you see a brown ring at the boundary between the two layers and a green layer on top, it indicates the presence of steroids.

5.1.2.4. Flavonoid detection methods:

i) Alkaline reagent test: Mix 1 ml of the test solution with a few drops of sodium hydroxide solution. A yellow color will appear, which will disappear when we add dilute HCl, showing the presence of flavonoids.

ii) Lead acetate Test: Add a few drops of lead acetate solution to 1 ml of the test solution in a test tube. A yellow precipitate will form, indicating the presence of flavonoids.

5.1.2.5. Terpenoid testing:

Salkowski Test: Mix 1 ml of the test solution with 2 ml of chloroform and a few drops of sulfuric acid. Shake well and let it sit for a while. A reddish-brown color will appear, indicating the presence of terpenoids.

5.1.2.6. Test for Saponins:

Froth test: Add 1 ml of the test solution to a test tube with water and shake it well. Check for a stable froth that lasts for at least 2 minutes, the formation of froth (foam) on the surface of the liquid that indicate the prescence of saponin.

5.1.2.7. Test for Phenols:

(i) Ferric chloride test: Add 1 ml of the extract solution to a test tube. Then add 1% gelatin solution. If a bluish-black color forms, it indicates the presence of phenols.

(ii) Lead acetate test: Add 1 ml of the extract solution to a test tube. Then add 1 ml of an alcoholic solution and dilute with 20% sulfuric acid. Finally, add sodium hydroxide. If the solution changes color from red to blue, it indicates the presence of phenols.

(b). Quantitative Methods:

Gas Chromatography- Microscopic Spectrometry Analysis (GC-MS):

This advanced and detailed analysis can be done using a GC MS-QP 2010 instrument with a DB-5 capillary column and helium as the carrier gas. The extracts inject at a specific or suitable temperature and subjected to a programmed oven temperature to analyse the sample for compounds present in it. The compounds in the extracts are identified by comparing the data with the NIST-MS, FFNSC Wiley Library, and existing literature reports, as well as using retention indices (RI). The extracts for analysis were inject at a specific temperature of 260 °C. The oven temperature included initially at 60°C, followed by a ramp of 3°C/min up to 210°C, then a ramp of 6°C/min up to 280°C (for 2 min), and at last held for 11 min.

The GC-MS analysis gave important information about the chemical makeup of the samples, helping to identify the different compounds in the extracts.

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